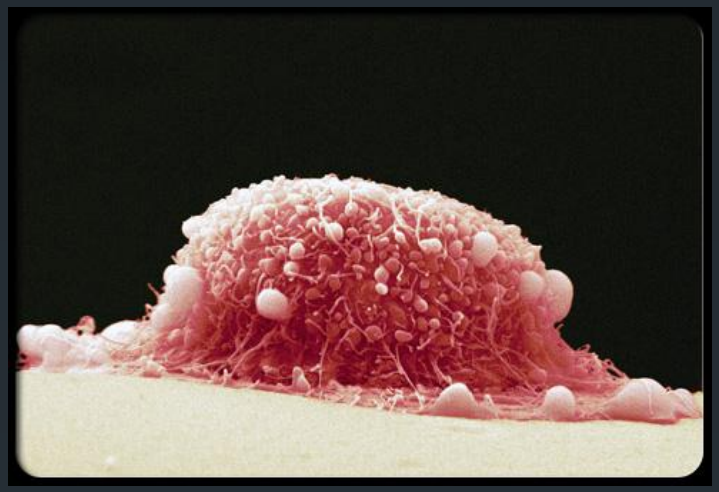


Cervical Cancer Screening
on the way to a shift from cytology to molecular

Presented BY: Siavash Bakhshian
Superwised BY: Dr M. Aslanimehr
Qazvin University of Medical Sciences
Faculty Of Medicine

Epidemiology

- Cervical cancer is the **second most prevalent cancer** seen in women worldwide
- about **500,000 cases** and over **270,000 deaths** estimated annually.
- cervical cancer is a **STD** that results from infection with certain high-risk, oncogenic types of the **human papillomavirus (HPV)**.
- HPV DNA is found **in more 99%** of invasive cervical cancers worldwide.

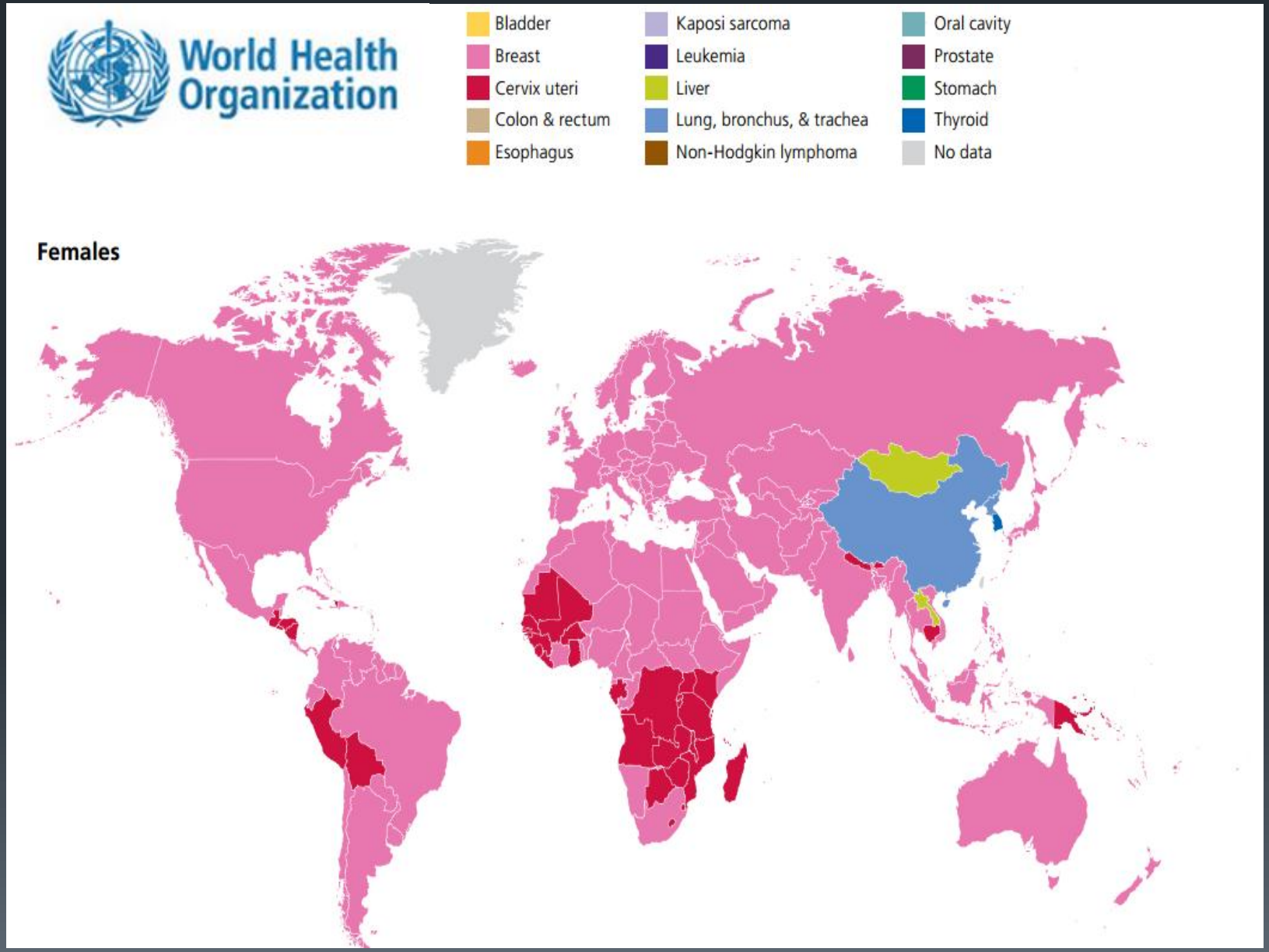
Table 1. Leading Causes of Death Worldwide by Income Level, 2012 (Thousands)

	Worldwide			Low- and Middle-income			High-income		
	Rank	Deaths	%	Rank	Deaths	%	Rank	Deaths	%
Cardiovascular diseases	1	17,513	31%	1	13,075	30%	1	4,438	38%
Malignant neoplasms	2	8,204	15%	3	5,310	12%	2	2,894	25%
Infectious and parasitic diseases	3	6,431	12%	2	6,128	14%	7	303	3%
Respiratory diseases	4	4,040	7%	4	3,395	8%	3	645	6%
Unintentional injuries	5	3,716	7%	5	3,212	7%	5	504	4%
Respiratory infections	6	3,060	5%	6	2,664	6%	6	396	3%
Digestive diseases	7	2,263	4%	7	1,748	4%	4	515	4%
Diabetes mellitus	8	1,497	3%	8	1,243	3%	9	254	2%
Intentional injuries	9	1,428	3%	9	1,185	3%	10	243	2%
Genitourinary diseases	10	1,195	2%	10	935	2%	8	260	2%
Nutritional deficiencies	11	559	1%	11	534	1%	14	25	0%
Congenital anomalies	12	556	1%	12	515	1%	13	42	0%
Maternal conditions	13	296	1%	13	293	1%	16	3	0%
Musculoskeletal diseases	14	216	0%	14	158	0%	12	58	1%
Other neoplasms	15	193	0%	15	116	0%	11	77	1%
All causes		55,843			44,172			11,671	

Source: World Health Organization Global Health Observatory Data Repository, Mortality and Global Health Estimates 2012. apps.who.int/gho/data/?theme=main. Accessed August 24, 2014.

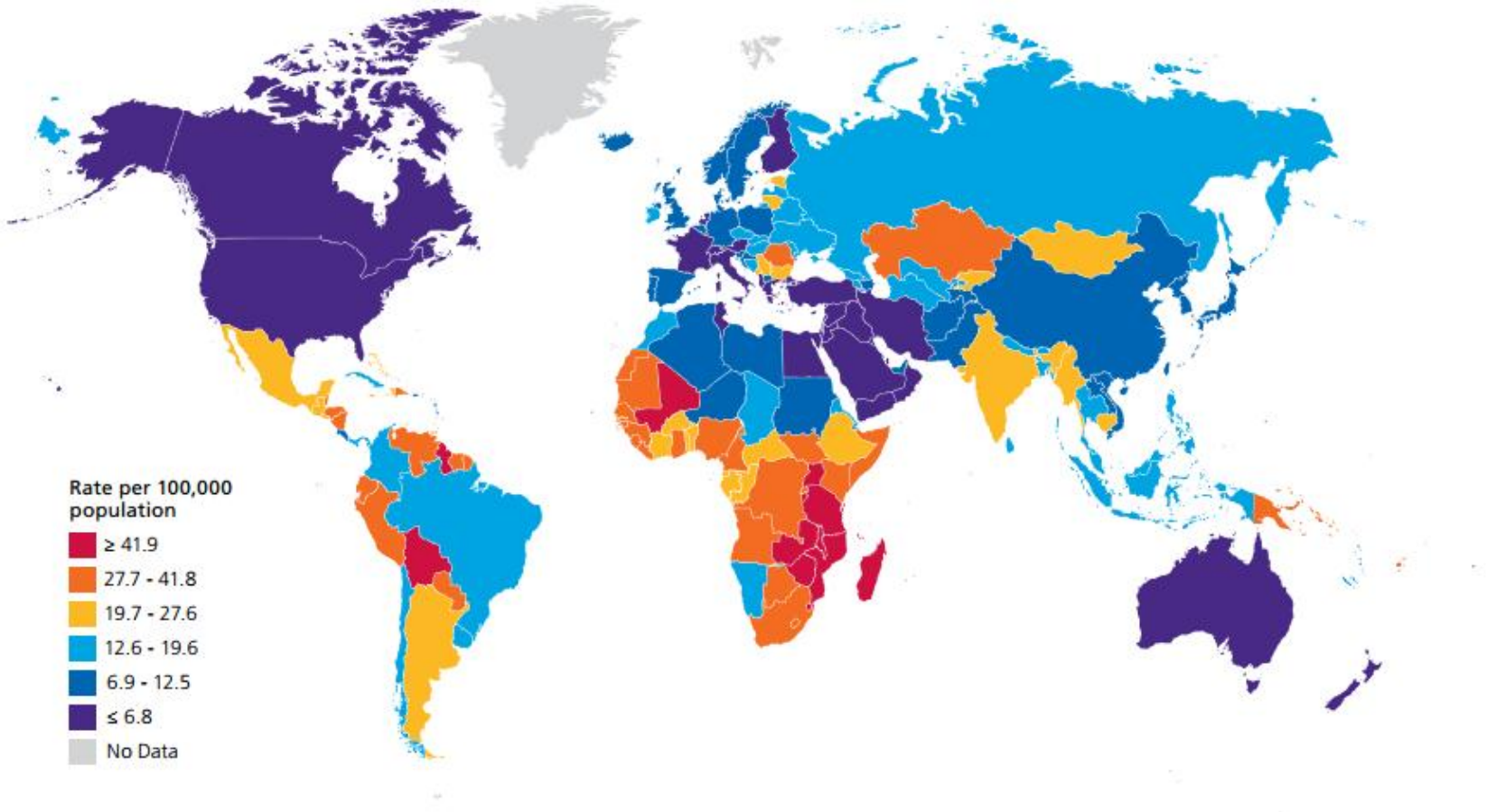
American Cancer Society, Inc., Surveillance Research, 2015

Dispersion of Females Dominant Cancers around the world 2012



World Health Organization

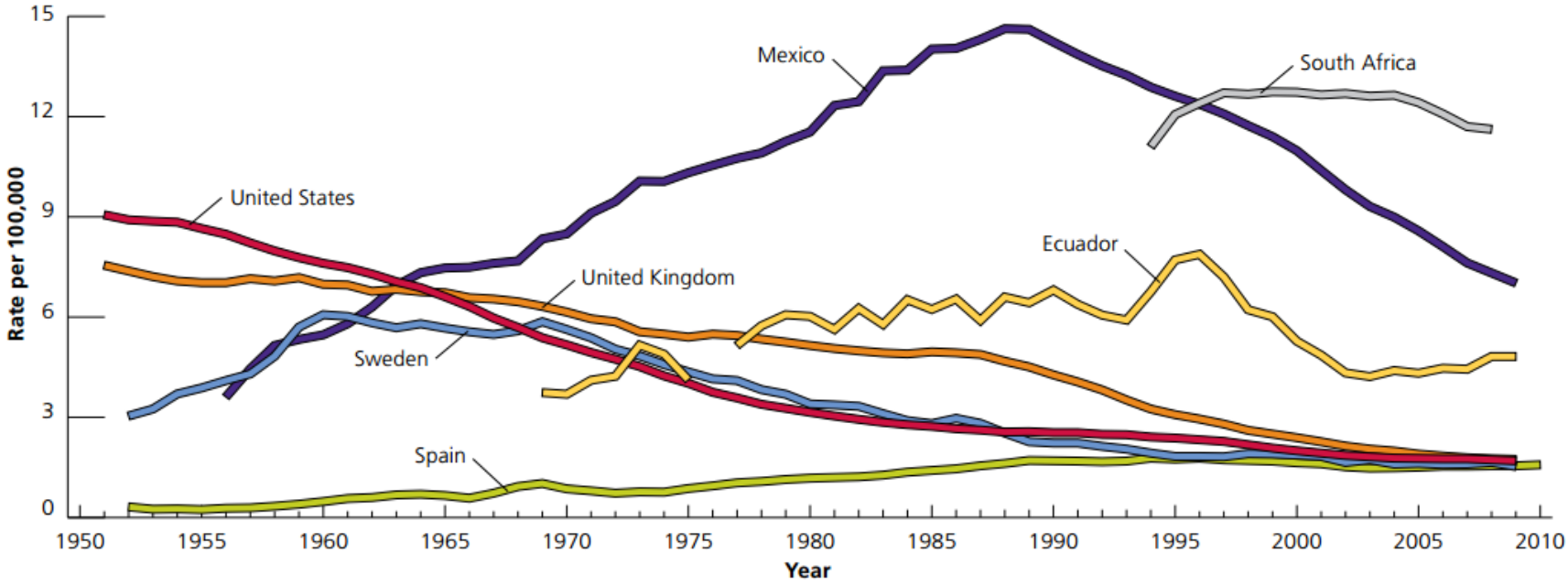
International Variation in Uterine Cervix Cancer Incidence Rates*, 2012



*Per 100,000, age standardized to the World Standard Population. **Source:** GLOBOCAN 2012.

World Health Organization

Trends in Cervical Cancer Death Rates* in Select Countries

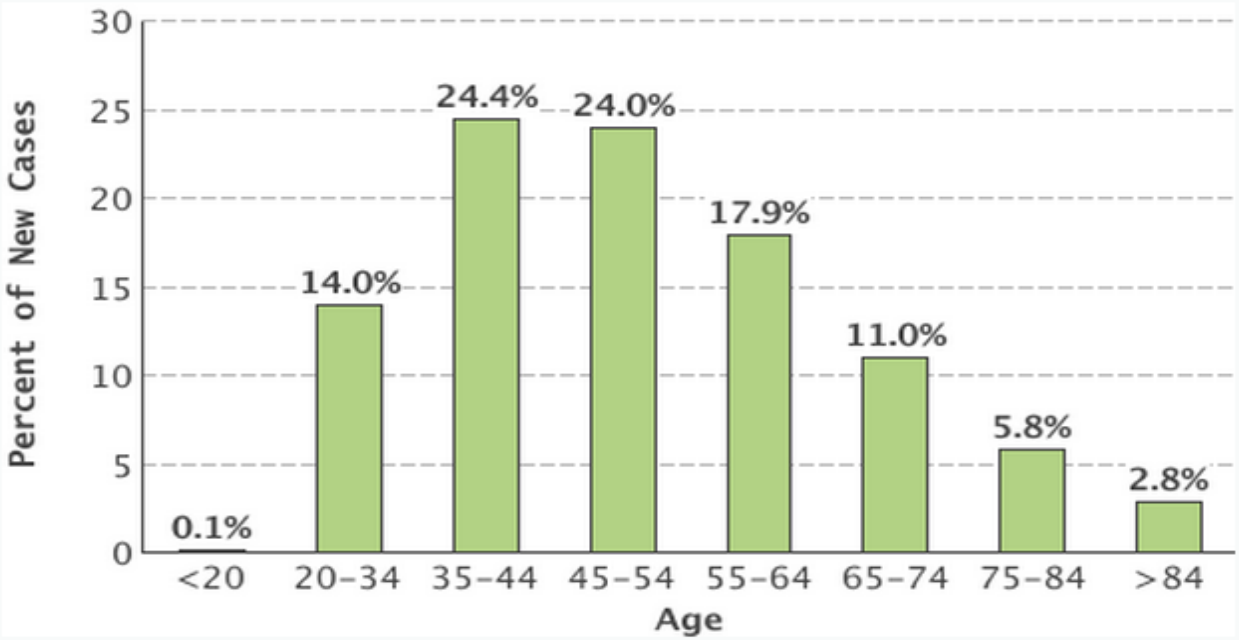


*Per 100,000, age standardized to the World Standard Population. Rates have been smoothed using 3-year average. Note: Break in trend indicates missing data.

Source: WHO Cancer Mortality Database.

Publication:2012

Percent of New Cases by Age Group: Cervix Uteri Cancer



Cervix uteri cancer is most frequently diagnosed among women aged 35–44.

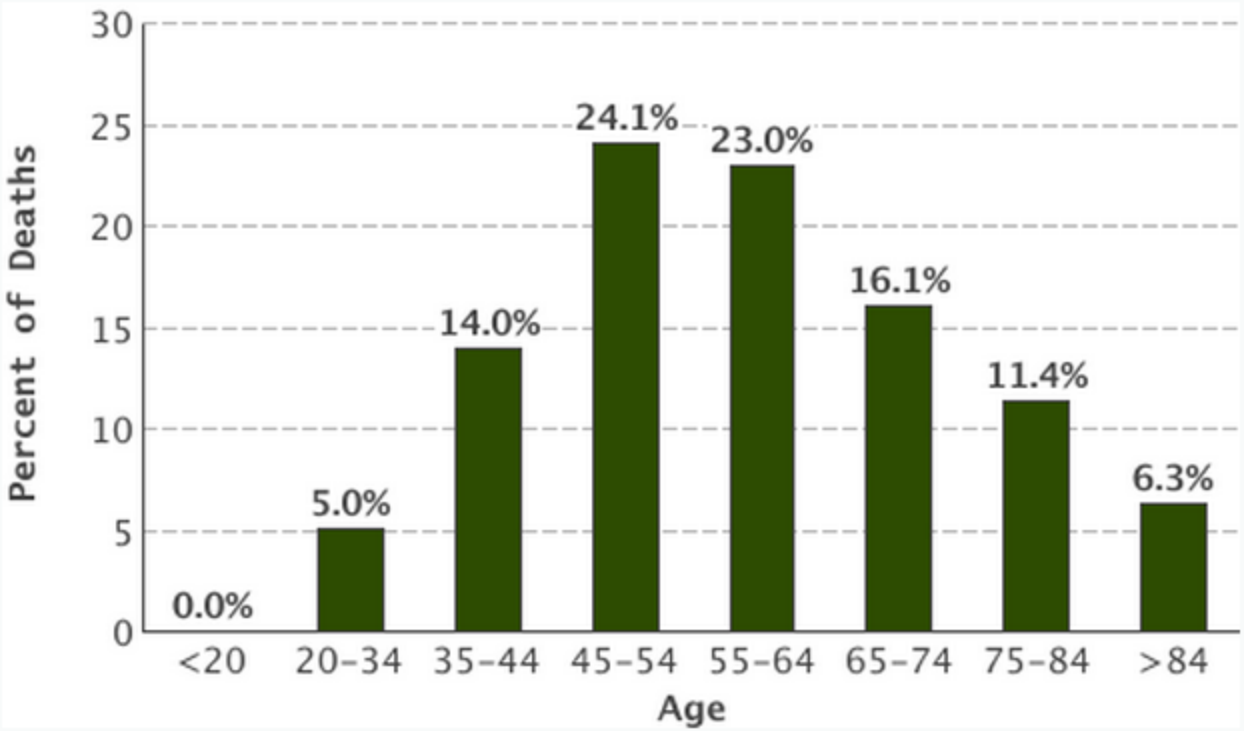
Median Age At Diagnosis

49

National Cancer Institute

2012

Percent of Deaths by Age Group: Cervix Uteri Cancer



The percent of cervix uteri cancer deaths is highest among women aged 45–54.

Median Age At Death
57

National Cancer Institute

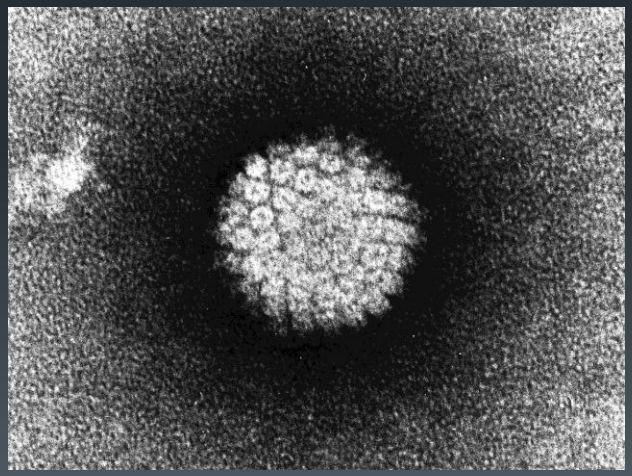
2012

The Main Agent:

- In recent years contamination with **oncogene viruses** (HPV-EBV-HBV-HCV…) is cause of **About 20% of cancers** all around the world.
- Role of **persistent infection with HPV** in creation of Cervical Cancer is fully known.
- In More 99% Cases of cervical cancer HPV virus diagnosed.

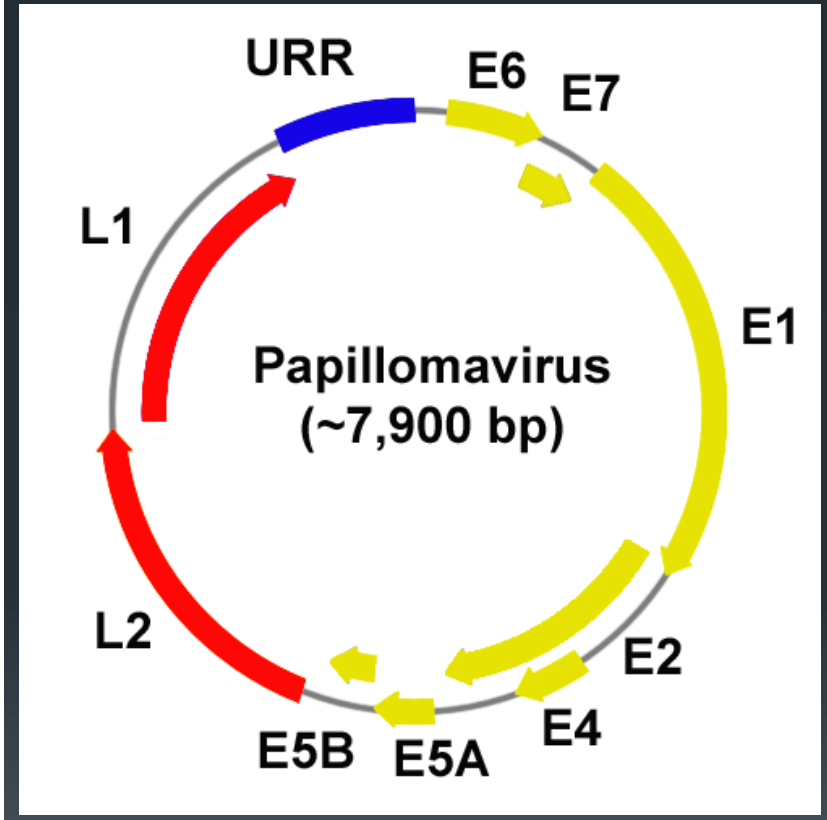
HPV : Human Papilloma Virus

- DNA Virus – ds Circular Genome
- Icosahedral Capsid Symmetry
- Naked Virion



HPV genes and their functions

HPV gene	Function
E1, E2	Viral replication
	Autoregulation of E1 and E2 function by E2
	Repression of E6 and E7 expression by E2
E5	Inhibition of apoptosis
	? Immune dysregulation
E6	p53 degradation
	Inhibition of apoptosis
	Cell cycle progression
	Cell transformation
	Immune dysregulation
E7	pRb inhibition
	Cell cycle progression
	Cell transformation
	Immune dysregulation
E4	? Facilitates release of mature viral particles
L1, L2	Synthesis of viral capsid
	Antigenicity



HPV Genotypes:

- **120 genotype of HPV identified** but Only some of them can progress to Cervical cancer (that named **High Risk** Genotypes)

- **16 And 18 are Most high risk genotypes** that cause of more than **70 %** invasive carcinoma.

- Other genotypes like **6 – 11 are low risk for Cervical cancer** (Not inconceivable) But main cause of **Genital Warts**.

HPV classification into high- and low-risk types	
Low-risk types	6, 11, 40, 42–44, 54, 61, 72, 81
High-risk types	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 82

HPV
30–40 Genital Types

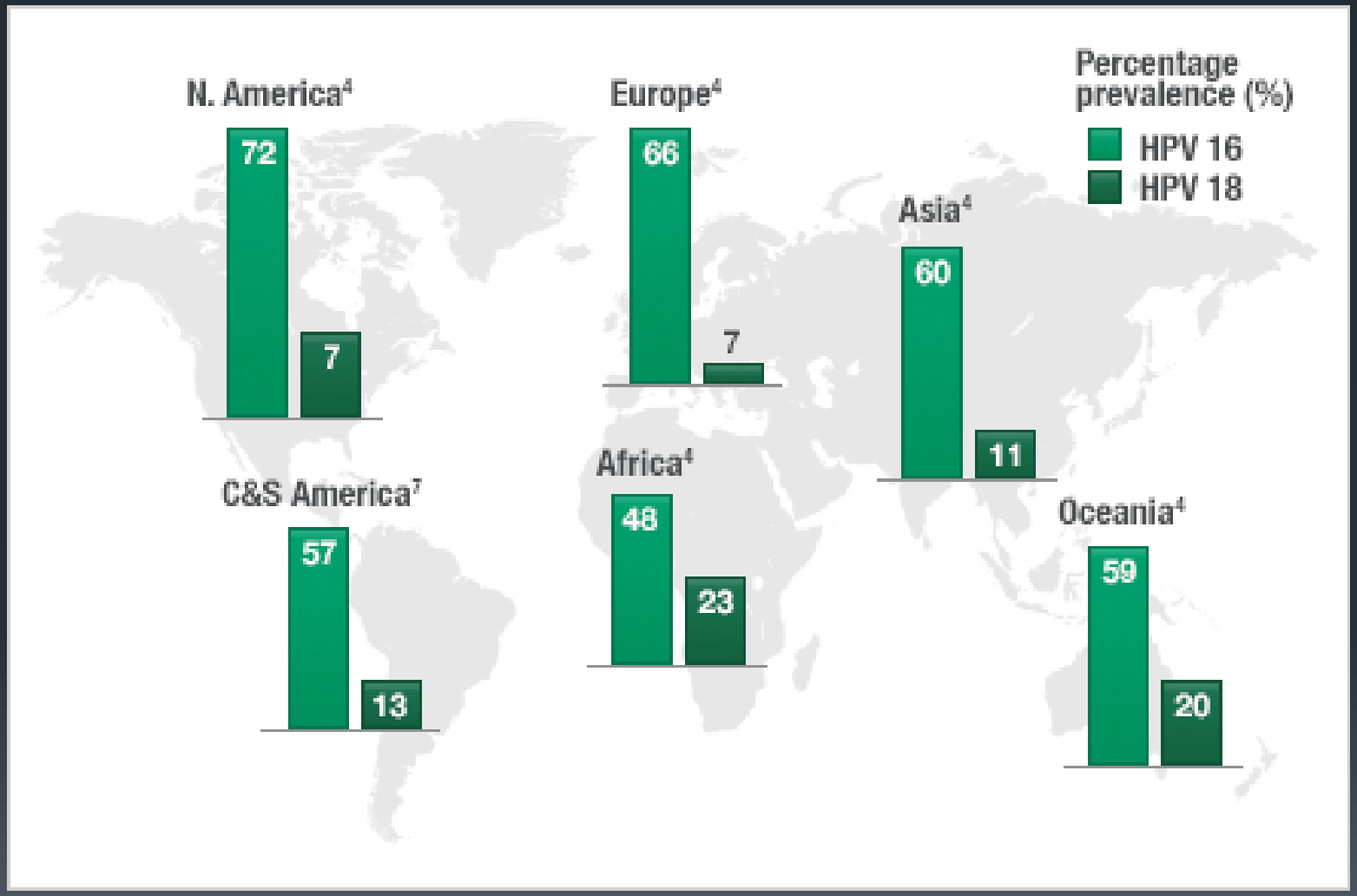
Types 6 and 11

cause 90% of
genital warts cases

Types 16 and 18

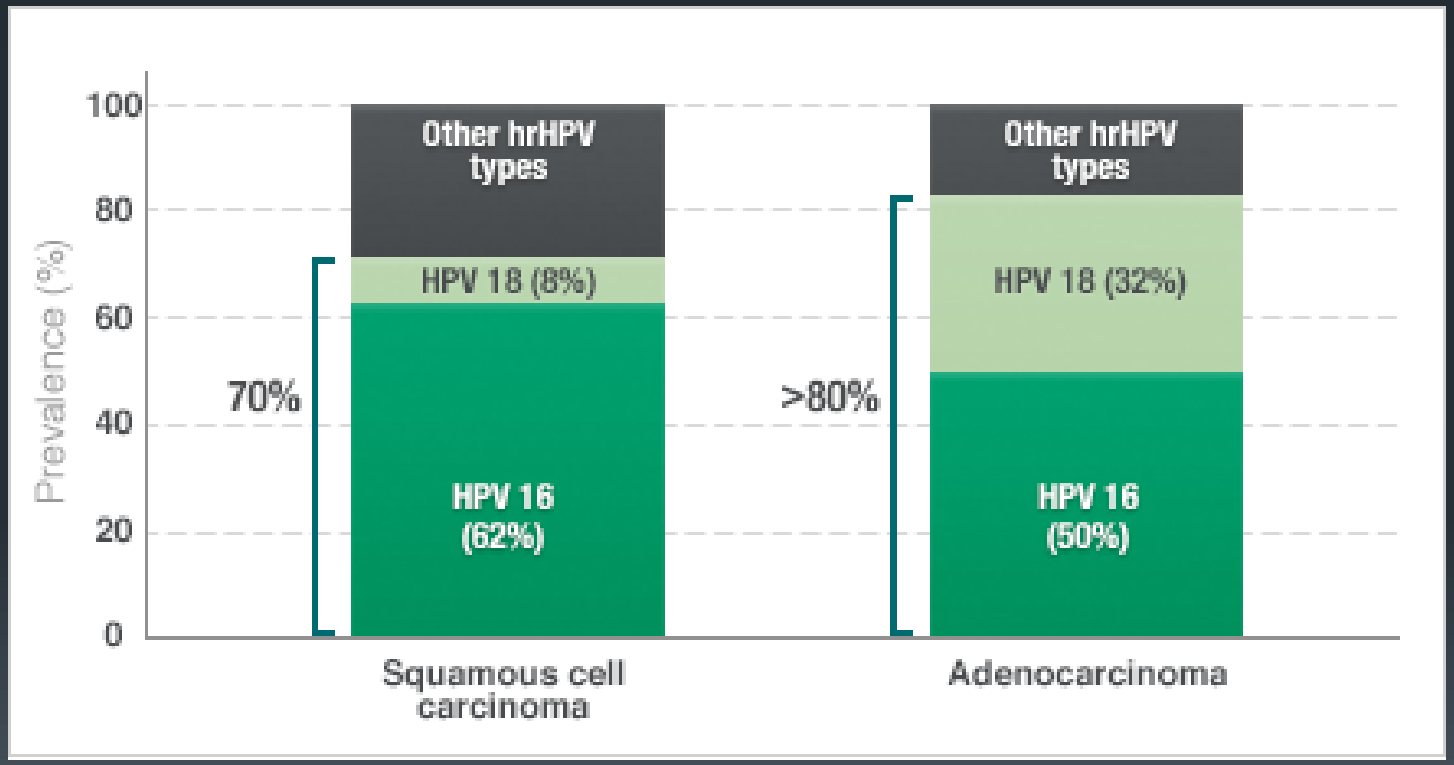
cause 70% of
cervical cancer cases
~40%–50% of vulvar cancer cases
~70% of vaginal cancer cases

Prevalence of HPV 16 & 18 Genotypes In World wide



de Sanjosé S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol.*2010;11(11):1048-1056.

HPV 16 and HPV 18 prevalence in squamous cell carcinoma and adenocarcinoma



de Sanjosé S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol.*2010;11(11):1048-1056.

Pathogenesis

- **persistent infection with an oncogenic HPV** is necessary for carcinogenesis

- In most Healthy women **immune response** to HPV infection develops after a period of months or years and results in adequate **viral clearance**.

- transformation is associated **with integration of the viral genome** and **epithelial cell genetic instability** that occurs over a long period of HPV infection.

Risk Factors:

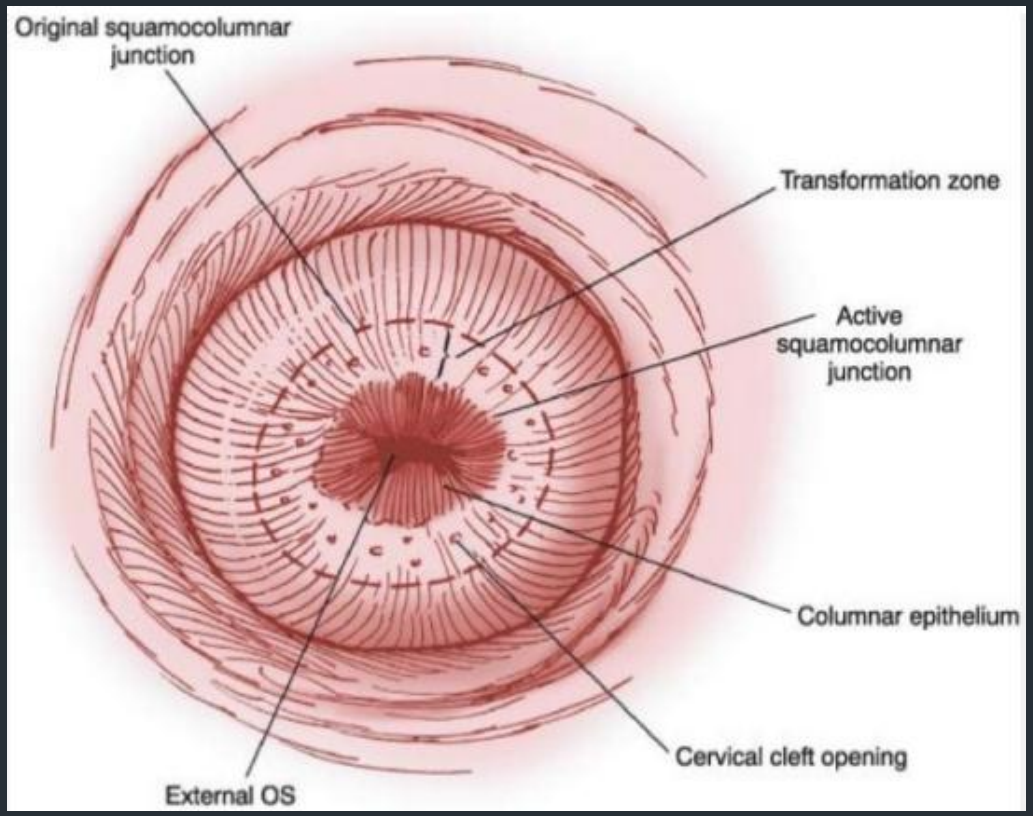
- early age of sexual activity
- multiple sexual partners
- exposure to other sexually transmitted diseases
- cigarette smoking
- oral contraceptive use
- human immunodeficiency virus (HIV)
- infection and immunosuppressive drug therapy.

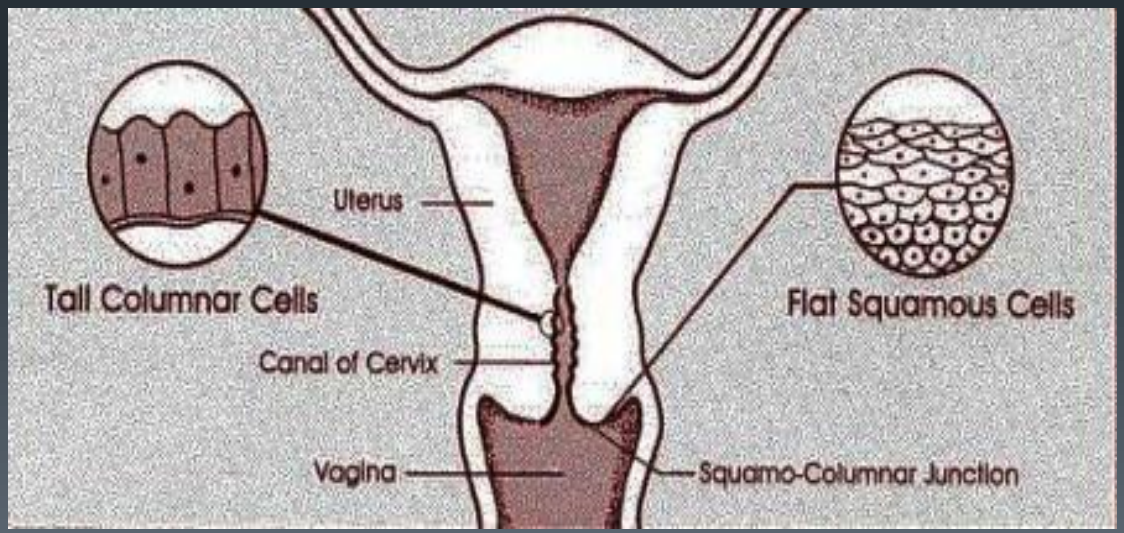
Clinical Aspects of HPV Infection

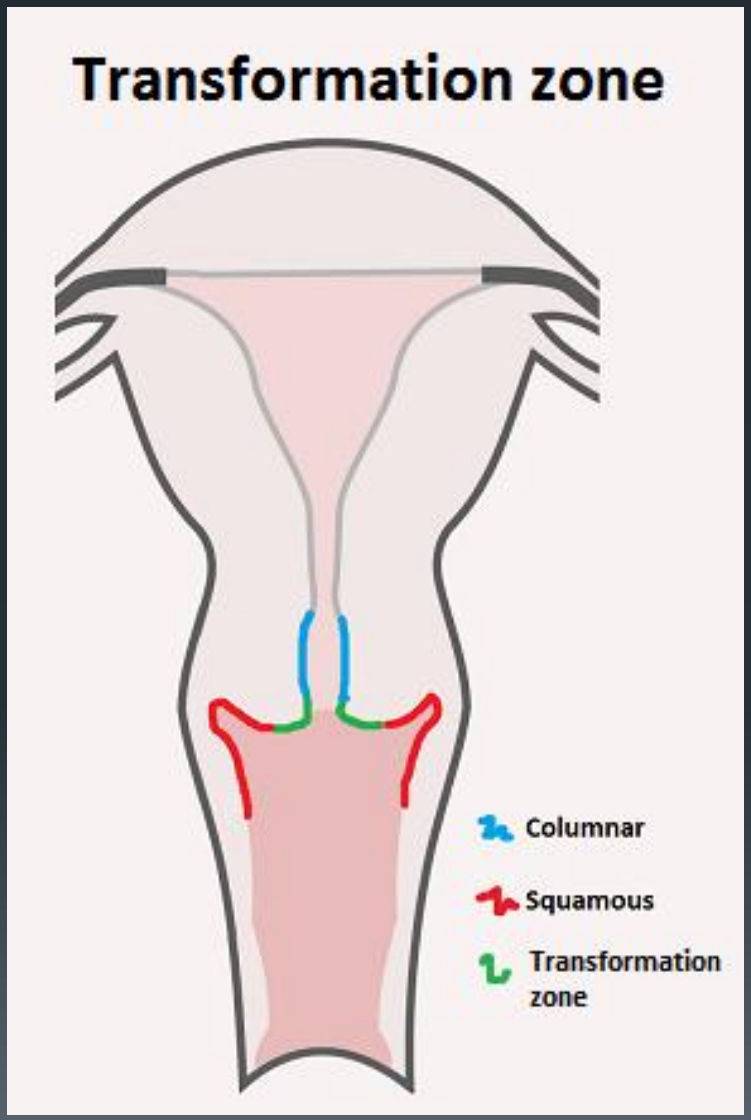
- The cervix normally contains **a central canal** (endocervical canal) that Contain **Columnar cells and squamous cells** lines.

- The squamo-columnar cellular junction is an active transition zone known as the **‘transformation zone’ (TZ)**.

- TZ is the site of origin of the majority of cervical dysplastic (premalignant) lesions and carcinomatous lesions.


Original squamocolumnar junction
Transformation zone
Active squamocolumnar junction
Columnar epithelium
Cervical cleft opening
External OS


Uterus
Tall Columnar Cells
Flat Squamous Cells
Canal of Cervix
Vagina
Squamo-Columnar Junction


Transformation zone
Columnar
Squamous
Transformation zone

Benign And Abnormal changes

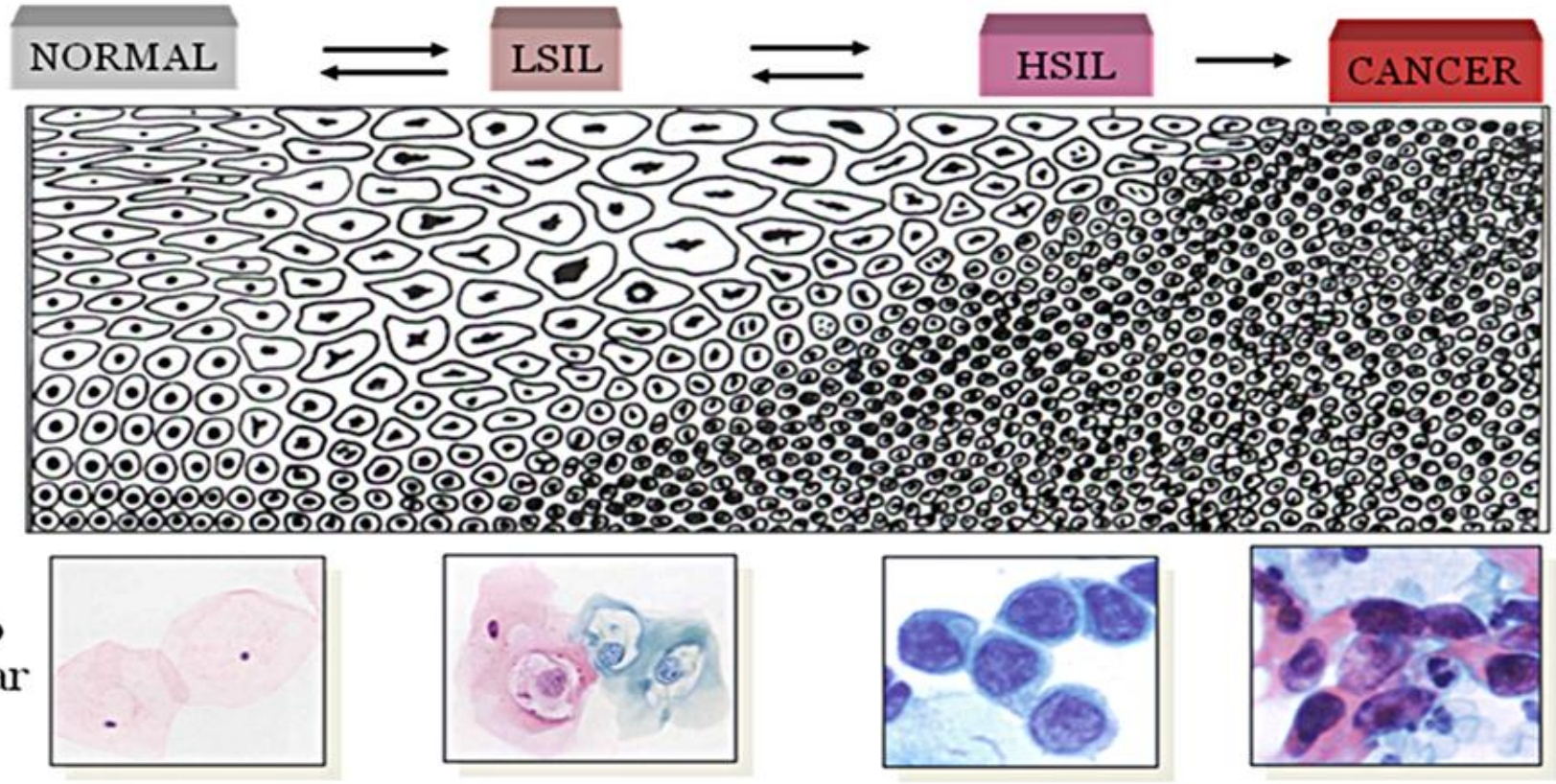
- In reproductive age women, the **acid environment** of the vagina leads to **destruction of the columnar cells** of the TZ, and induces ‘squamous metaplasia’ in the TZ.
- This metaplastic change clinically considered as **benign**.
- HPV infection can induce change **from normal to dysplastic** cellular architecture (clinically considered as abnormal) in the TZ and surrounding cervical portion.

Pap Smear : First Step of Diagnosis

- Cervical cancer screening involves the initial step of **accessing squamous cells in the transformation zone, for cytological analysis** (Papanicolau smear or Pap smear).

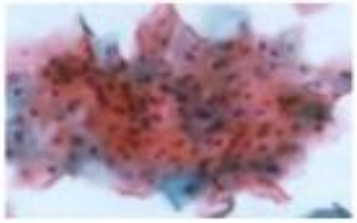
- Cervical cytology reports classify dysplastic lesions as:
  - low grade squamous intraepithelial lesions (LSIL)
  - high-grade squamous intraepithelial lesions (HSIL)
  - Squamous Cell Carcinoma

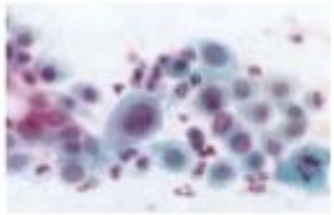
((according to the Bethesda system))


NORMAL
LSIL
HSIL
CANCER
Pap smear

General Categorization of Squamous cell lesion

- **Atypical squamous : ASC-US, ASC-H**

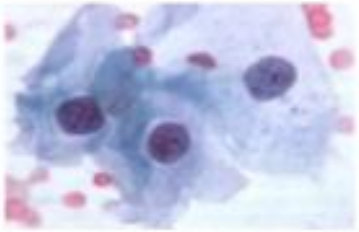




ASC-H

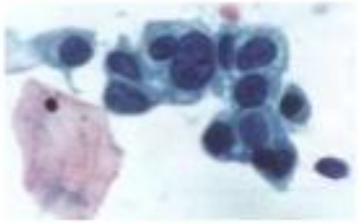
- LSIL

  (Low-grade squamous intraepithelial lesion)

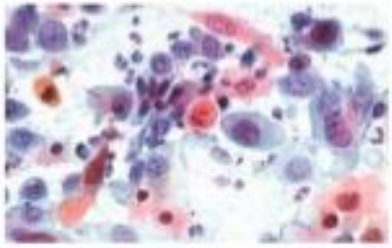


- HSIL

  (High-grade squamous intraepithelial lesion)



- Squamous cell carcinoma



ASC-US : atypical squamous cells of undetermined significance ;
ASC-H : atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion

Types of Screening

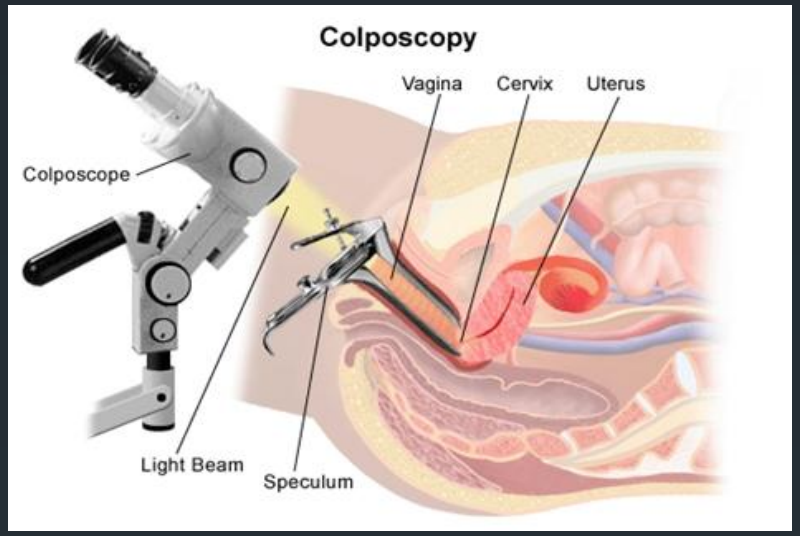
- Conventional Pap: In a conventional Pap smear, samples are smeared directly onto a microscope slide after collection and applies a fixative. In general, the slide is sent to a laboratory for evaluation.

- Liquid based cytology: The sample of epithelial cells is taken from the Transitional Zone. Liquid-based cytology uses an arrow-shaped brush, rather than the conventional spatula. The cells taken are suspended in a bottle of preservative for transport to the laboratory, where using Pap stains it is analysed.(Since the mid-1990s)

Liquid-based cytology

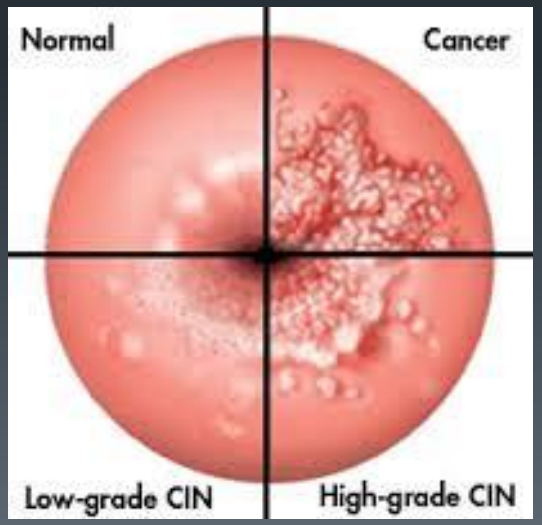
- Two of the types are Sure-Path (TriPath Imaging) and Thin-Prep (Cytyc Corp).
- The media are primarily ethanol-based for Sure-Path and methanol for ThinPrep.
- Once placed into the vial, the sample is processed at the laboratory into a cell thin-layer, stained, and examined by light microscopy.
- The liquid sample has the advantage of being suitable for high-risk HPV testing and may reduce unsatisfactory specimens from 4.1% to 2.6%.
- Proper sample acquisition is crucial to the accuracy of the test, as a cell that is not in the sample cannot be evaluated.

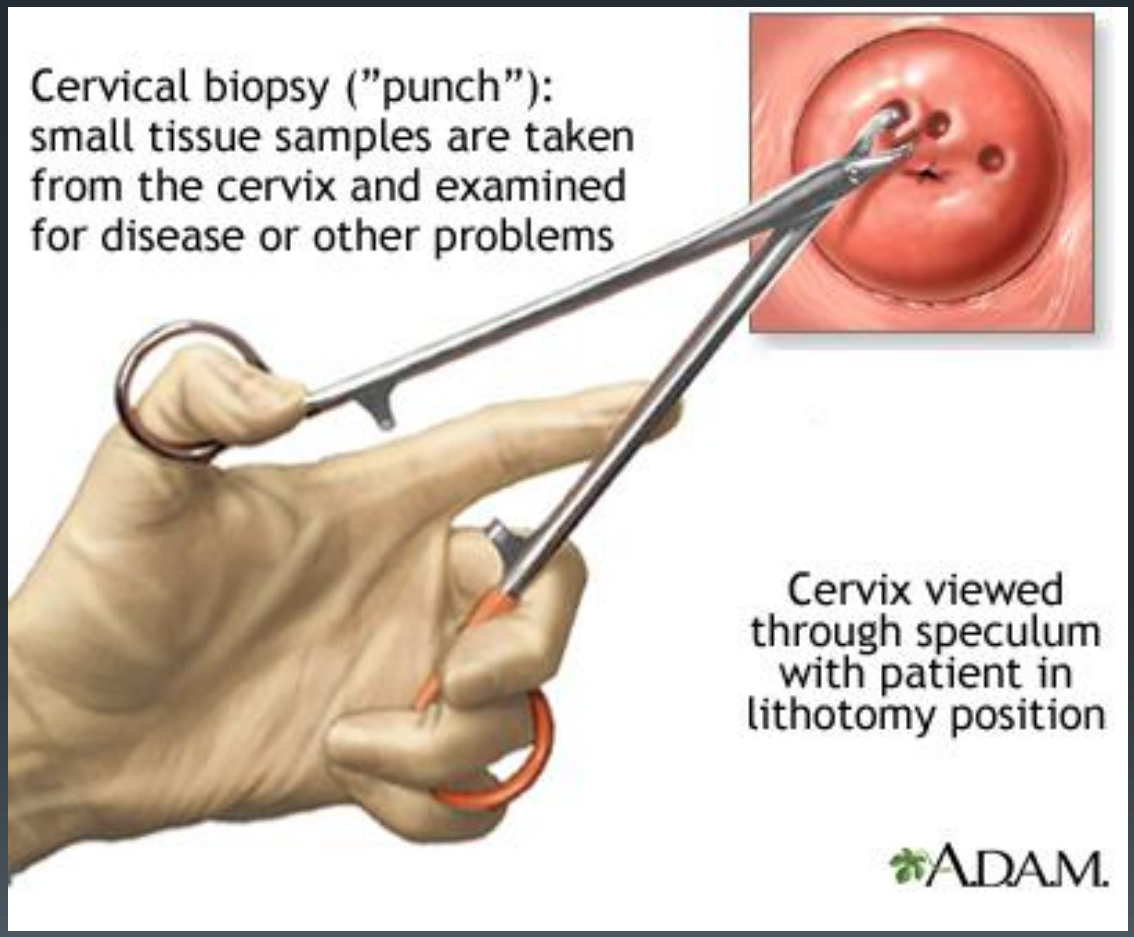
The next step:



- the next step following a Pap smear is the **assessment of the cervix using a microscope** (colposcopy).

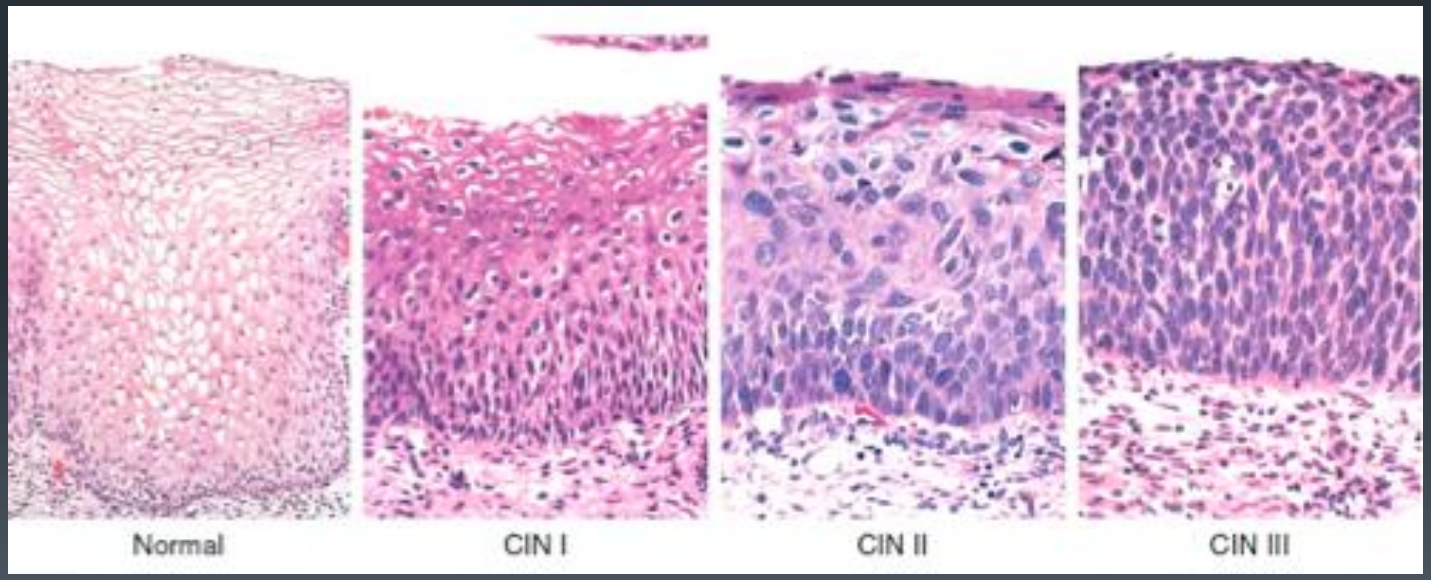
- Colposcopy usually **facilitates the obtaining of biopsies** from the cervical lip and endocervical canal.




Cervical biopsy ("punch"): small tissue samples are taken from the cervix and examined for disease or other problems
Cervix viewed through speculum with patient in lithotomy position
A.D.A.M.

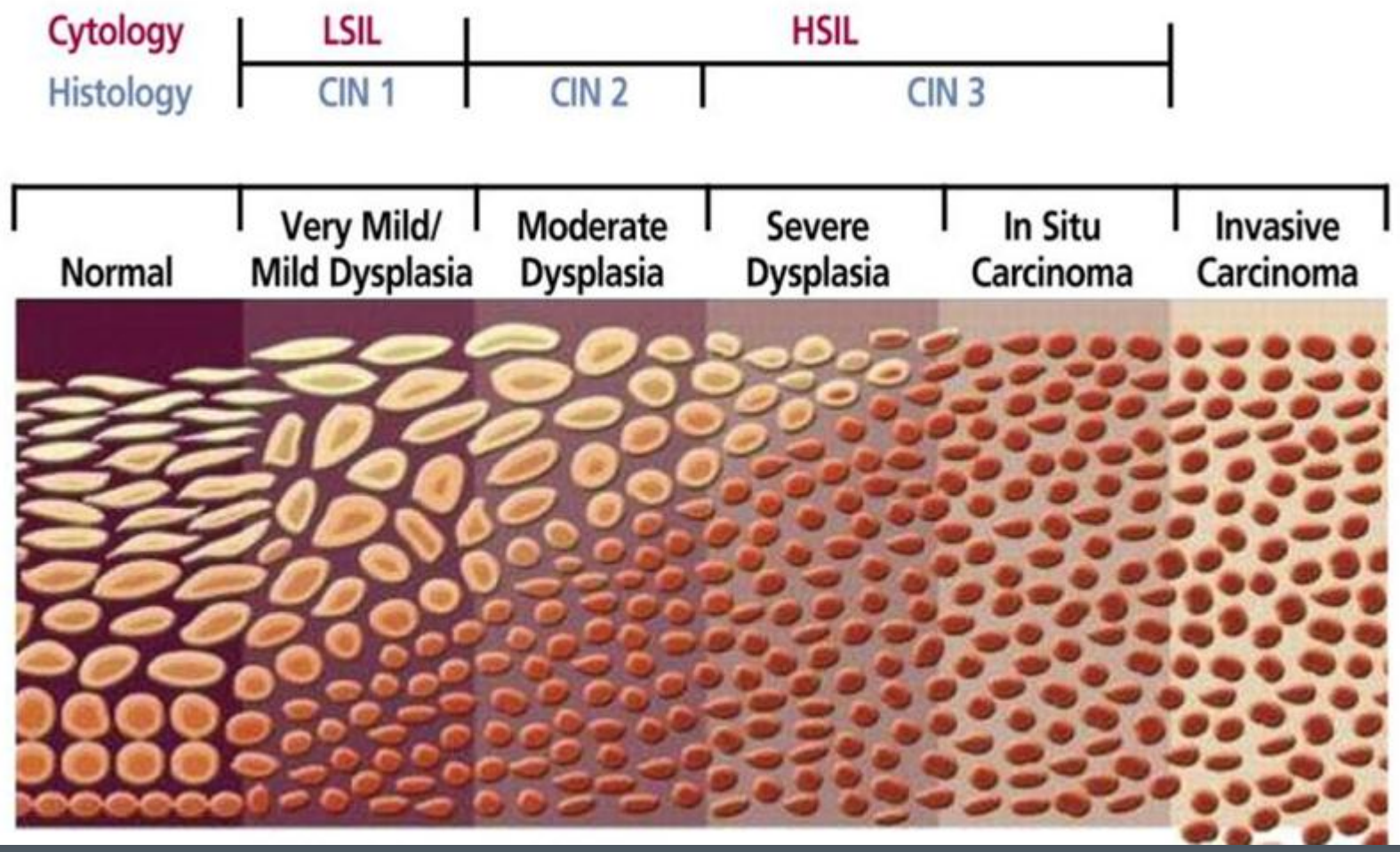
Next Step: Histology

- Histological reports of colposcopic biopsy specimens are reported as CIN 1, 2 or 3.



CIN : Cervical Intra epithelial Neoplasia

Comparison between Cytology and Histology Grading



Cytology limitations:

1- The major problem is the **low sensitivity of a single smear** to detect high-grade precursor lesions (50%–70%), which require frequent testing.

Cuzick J, Clavel C, Petry KU et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer 2006; 119: 1095–1101

Cytology limitations:

2- cytology has low reproducibility, leading to **variable accuracy**

Nanda K, McCrory DC, Myers ER et al. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med 2000; 132: 810–819.

Cytology limitations:

- 3- Adenocarcinoma of the cervix has not been shown to be prevented by Pap tests. Adenocarcinoma accounts for about 15% of all cervical cancers.

DeMay, M. (2007). *Practical principles of cytopathology. Revised edition*. Chicago, IL: American Society for Clinical Pathology Press. ISBN 978-0-89189-549-7.

Fail To Prevention …

Failure of prevention of cancer by the Pap test can occur for many reasons:

- not getting regular screening
- lack of appropriate follow up of abnormal results
- sampling and interpretation errors
- variable accuracy because of clinician direct diagnosis

Pap Test at best…

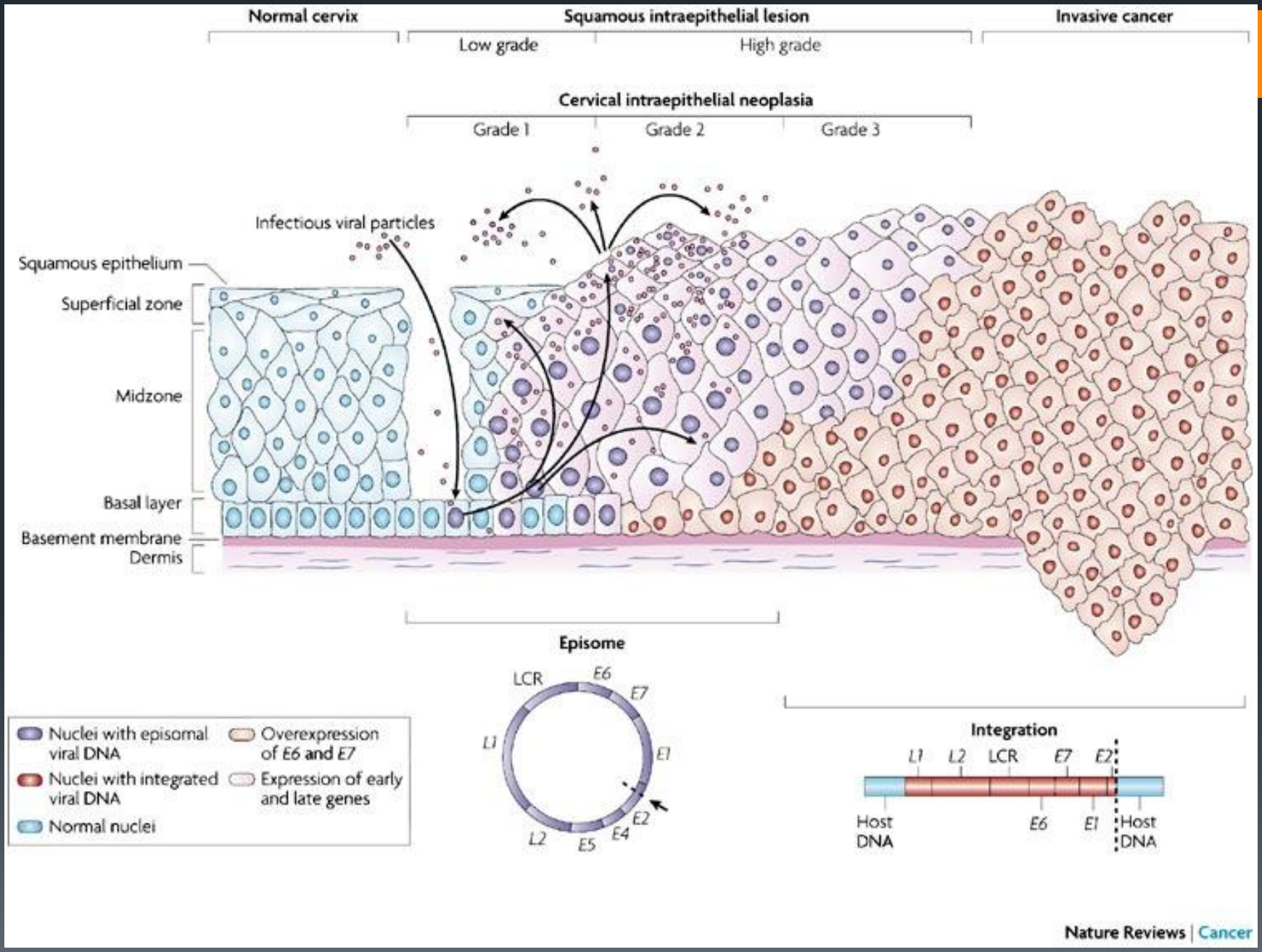
- The Pap test, when combined with a regular program of screening and appropriate follow-up, can **reduce cervical cancer deaths by up to 80%.**

Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U, Segnan N, Wiener H, Herbert A, von Karsa L (2010). "European Guidelines for Quality Assurance in Cervical Cancer Screening. Second Edition—Summary Document". *Annals of Oncology* **21** (3): 448–458. doi:10.1093/annonc/mdp471. PMC 2826099.PMID 20176693.

Molecular Science Biomarkers

The **HPV life cycle** and **molecular events** leading to cellular transformation, have provided insight into **potential biomarkers** that can be used as **adjunctive tests** to improve **diagnostic accuracy** of cervical lesions as well as, identify those patients at risk for progression to cancer.

Caner Cycle

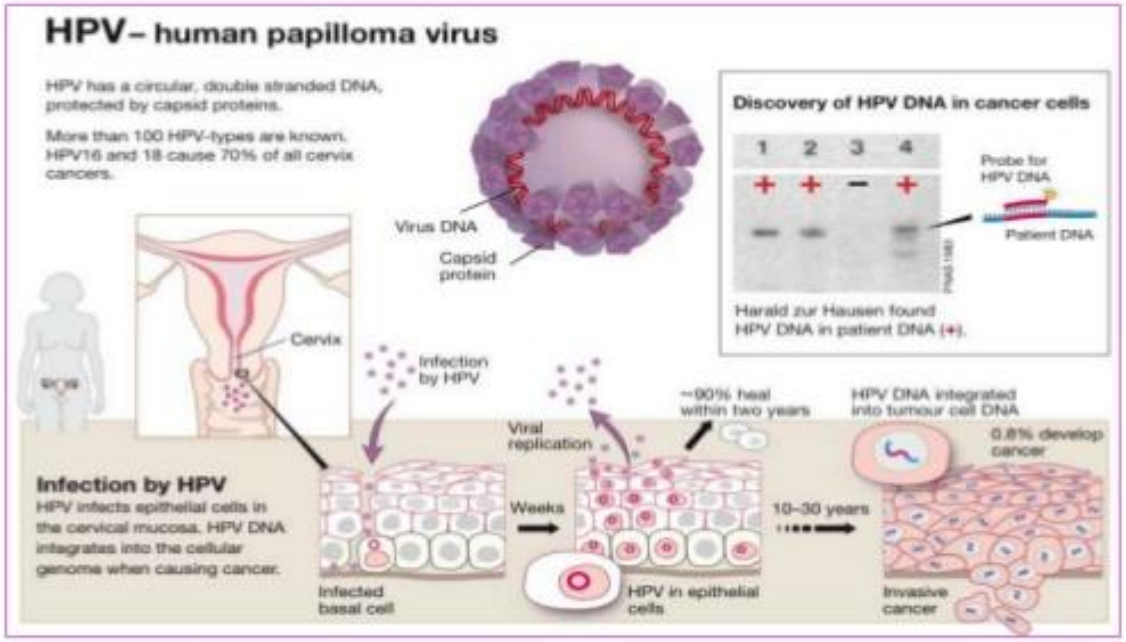

Normal cervix
Squamous intraepithelial lesion
Low grade
High grade
Invasive cancer
Cervical intraepithelial neoplasia
Grade 1
Grade 2
Grade 3
Infectious viral particles
Squamous epithelium
Superficial zone
Midzone
Basal layer
Basement membrane
Dermis
Episome
LCR
E6
E7
L1
E1
E2
L2
E5
E4
Integration
L1
L2
LCR
E7
E2
Host DNA
E6
E1
Host DNA
Nuclei with episomal viral DNA
Nuclei with integrated viral DNA
Normal nuclei
Overexpression of E6 and E7
Expression of early and late genes
Nature Reviews | Cancer

HPV DNA Testing

- The causal relationship between **infection with HR (High Risk) HPV** and **cervical cancer** has stimulated the application of hrHPV DNA testing
- which has been proposed, **either alone** or **in combination with cytology**, as a means to improve existing cervical screening programs
- The **most widely used and extensively investigated biomarker** in the management of cervical disease is HPV DNA testing.

HPV Life Cycle and Infection

❖The HPV life cycle consists of initial infection, uncoating, genome maintenance, genome amplification, and packaging to form new viral particles



- In the past 15 years, large randomized trials designed to evaluate the performance of hrHPV testing have provided important arguments for the implementation of this assay as a primary screening tool.

Trials comparing cytology and hrHPV testing in cervical cancer screening

Study	Description	Interval	Reference
POBASCAM	HPV (GP5+/6+-PCR) and cytology versus cytology alone	5 years	Bulkmans et al. [24, 109] Rijkaart et al. [33]
ARTISTIC	HPV (HC2) combined with cytology (LBC) versus cytology (LBC) alone	3 years	Kitchener et al. [25, 110]
SwedeScreen	HPV (GP5+/6+-PCR) and cytology versus cytology alone	3–5 years (by age)	Naucler et al. [26, 27]
NTCC	HPV (HC2) alone versus HPV (HC2) and cytology (LBC) versus cytology alone	3 years	Ronco et al. [28, 111, 112]
CCCaST	HPV (HC2) and cytology versus cytology and HPV (HC2) (randomized order of collection)	1 year	Mayrand et al. [29]
Finnish screening trial	HPV (HC2) and cytology triage versus cytology alone	5 years	Leinonen et al. [113]
India screening trial	HPV (HC2) versus cytology versus visual inspection with acetic acid (VIA) versus no screening	–	Shankaranarayanan et al. [41]

Mayrand 2007- Ronco 2008 - Naucler 2007 - Leinonen 2009 - Kitchener 2009 - Rijkaart 2012

Commercial Available DNA Assays

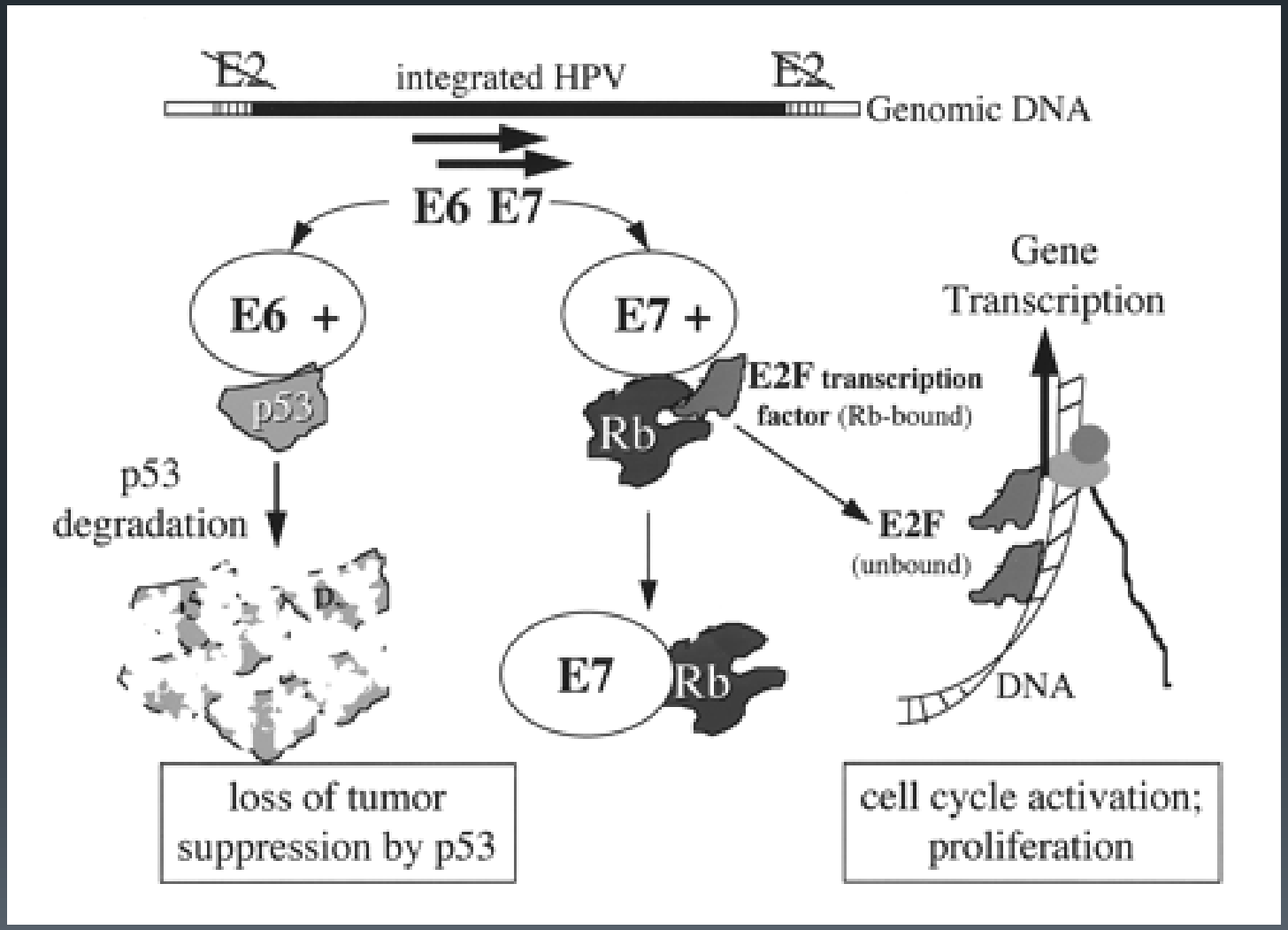
Available assays	Manufacturer	Target	HPV genotypes	Genotyping	FDA approved
Viral Assay HPV DNA					
COBAS 4800	Roche	L1 DNA	13 HR HPV and HPV66	16 and 18	Yes
Cervista	Hologic	L1 DNA	13 HR HPV and HPV66	16 and 18	Yes
Hybrid Capture 2	QIAGEN	Full Genome	13 HR HPV and HPV66	No	Yes
Amplicor	Roche	L1 DNA	13 HR HPV	No	No
CareHPV	QIAGEN	L1 DNA	13 HR HPV and HPV66	No	No
Digene HPV eHC	QIAGEN	Full Genome	13 HR HPV, HPV66 and 82	No	No
EIA kit HPV GP HR	Diassay	L1 DNA	13 HR HPV and HPV66	No	No
INFINITI HPV-HR QUAD	AutoGenomics	E1 DNA	13 HR HPV and HPV66	No	No
RT HPV	Abbott	L1 DNA	13 HR HPV and HPV66	16 and 18	No
Digene HPV eHC 16 18/45	QIAGEN	Full Genome	13 HR HPV, HPV66 and 82	16, 18, and 45	No
Clart	Genomica	L1 DNA	13 HR HPV and 22 no HR	Yes	No
INFINITITM	Genomica	L1 DNA	13 HR HPV and 12 no HR	Yes	No
InnoLiPA	Innogenetics	L1 DNA	13 HR HPV and 15 no HR	Yes	No
Linear Array	Roche	L1 DNA	13 HR HPV and 24 no HR	Yes	No
Multiplex HPV genotyping	Multimetrix	L1 DNA	13 HR HPV and 11 no HR	Yes	No
PapilloCheck	Greiner Bio-One	E1 DNA	13 HR HPV and 11 no HR	Yes	No

More advanced Biomarkers…

Promising biomarkers involved in cervical carcinogenesis have already emerged.

Especially, biomarkers that indicate a shift from the productive phase of hrHPV infection to the transforming phase are valuable.

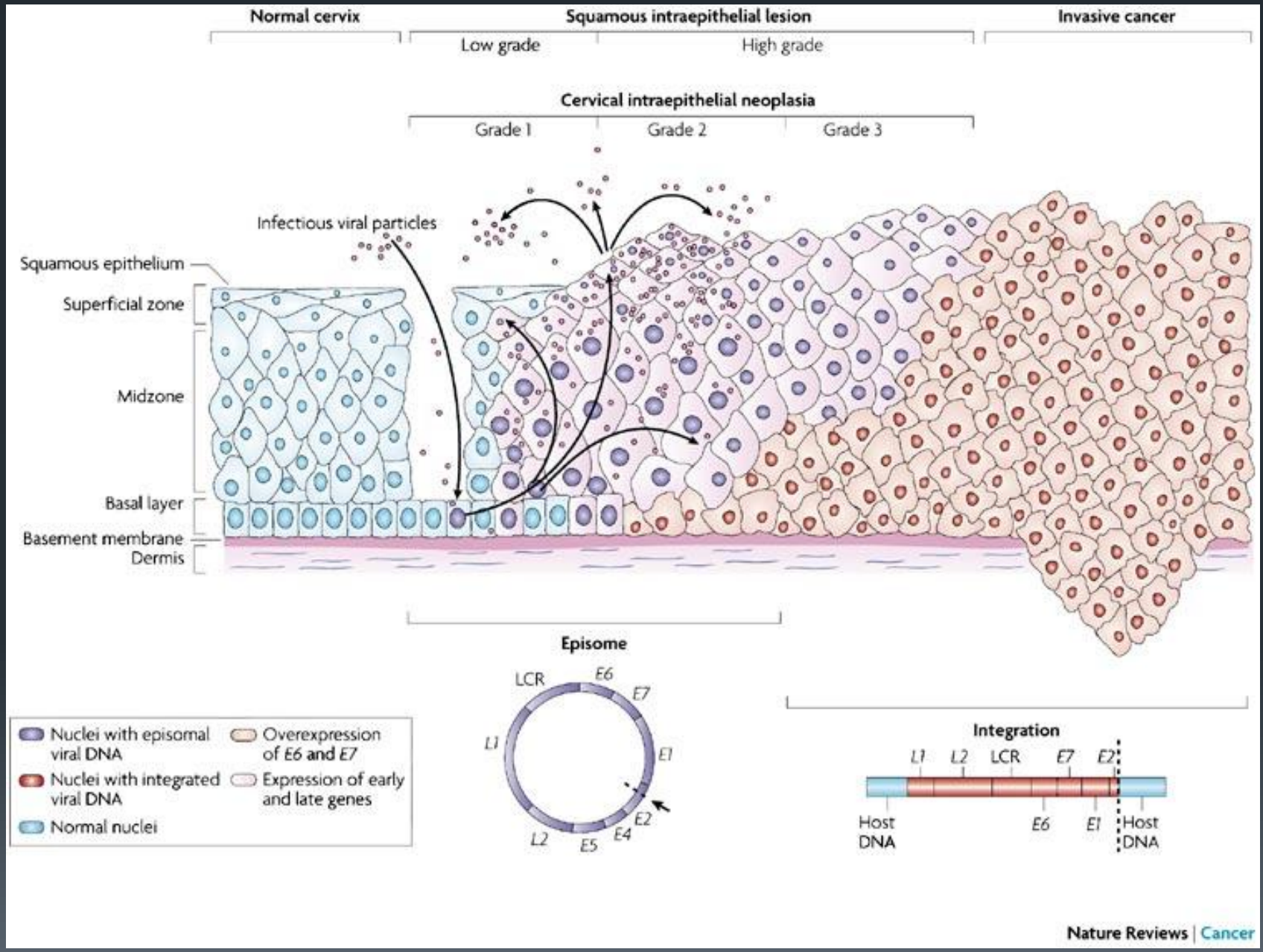
E6-E7 Proteins Function



E6/E7 mRNA as a Biomarker

- HPV mRNA assays are designed to detect viral mRNAs encoding for the **E6 and E7** proteins which are the **most critical factors for the development of cervical cancer**.

- Therefore, **detection of elevated E6/E7 mRNA levels** in cervical smears has been suggested to be an **attractive biomarker**


Normal cervix
Squamous intraepithelial lesion
Low grade
High grade
Invasive cancer
Cervical intraepithelial neoplasia
Grade 1
Grade 2
Grade 3
Infectious viral particles
Squamous epithelium
Superficial zone
Midzone
Basal layer
Basement membrane
Dermis
Episome
LCR
E6
E7
E1
E2
E4
E5
L2
L1
Integration
L1
L2
LCR
E7
E2
E6
E1
Host DNA
Host DNA
Nuclei with episomal viral DNA
Nuclei with integrated viral DNA
Normal nuclei
Overexpression of E6 and E7
Expression of early and late genes
Nature Reviews | Cancer

Commercial Available mRNA Assays

Available assays	Manufacturer	Target	HPV genotypes	Genotyping	FDA approved
HPV RNA					
APTIMA	GenProbe	E6/E7 mRNA	13 HR HPV and HPV66	No	Yes
NucliSens EasyQ	Biomerieux	E6/E7 mRNA	5 HR HPV	16, 18, 31, 33, and 45	No
OncoTect	IncellDx	E6/E7 mRNA	13 HR HPV	Yes	No
PreTect Proofer	Norchip	E6/E7 mRNA	5 HR HPV	16, 18, 31, 33, and 45	No

Cellular Biomarkers

- An alternative is the **detection of cellular host genes** that are specifically **up-regulated and overexpressed, or silenced** in cells that have undergone the shift into the transforming phase of hrHPV infection.

- The functional inactivation of p53 and pRb oncosuppressors by E6 and E7 oncoproteins determines the alteration of several cellular pathways relevant for cell transformation and cancer development.

p16INK4a

- p16INK4a is a **tumor-suppressor protein** that inhibit transcription and cell-cycle progression. In most cervical carcinomas, the functional inactivation of pRb by HPV E7 results in the **overexpression of p16INK4a and the accumulation of the protein in cells**.

Why p16INK4a is Suitable?

- (1) expression of p16INK4a is directly linked to the HPV oncogenic action and **start of oncogenes**.
- (2) the expression of p16INK4a is independent of the HPV type, and therefore, **genotyping does not need**

Ki-67

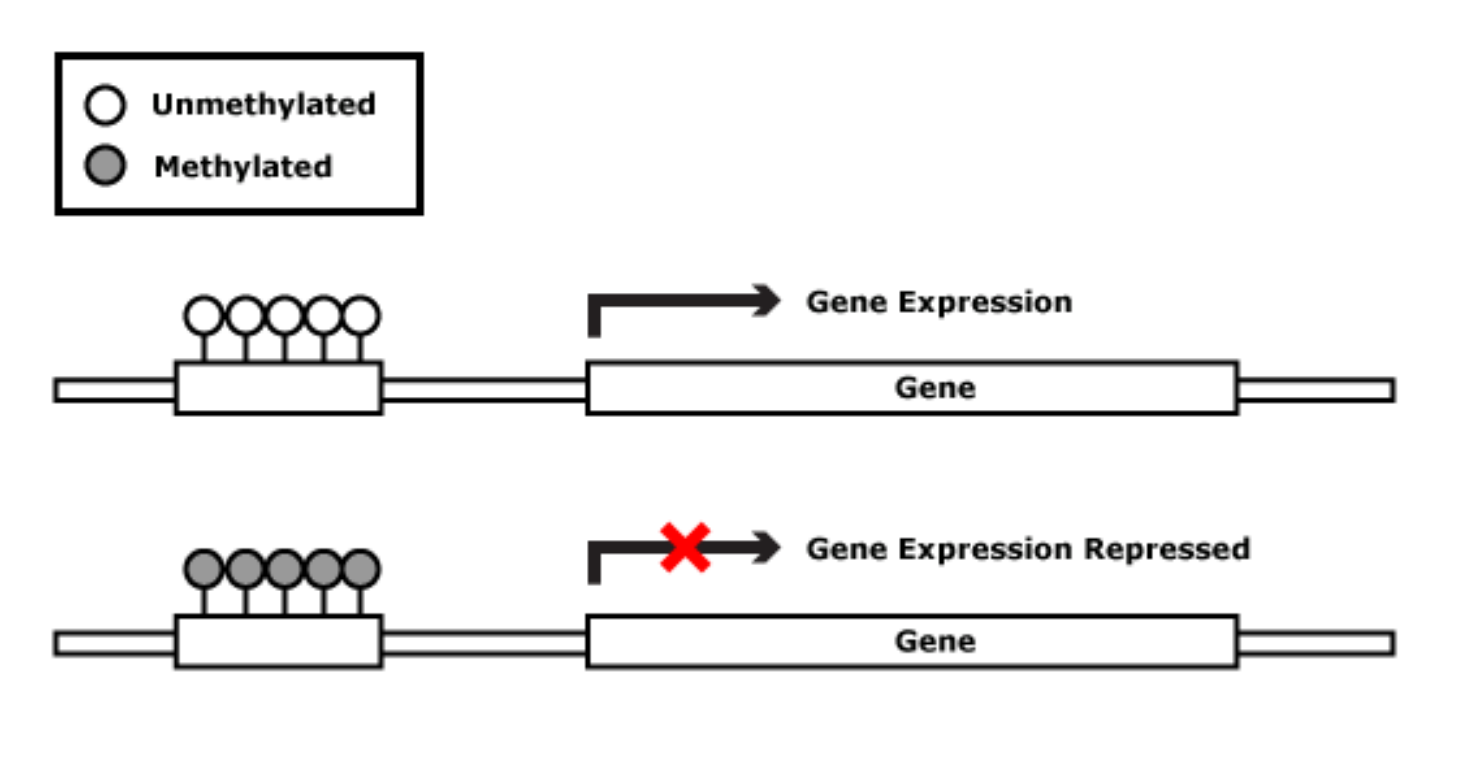
- The **proliferation antigen Ki-67**,which is **expressed during the G2 and mitotic phases of the cell cycle**, has been demonstrated in many studies to be a reliable indicator of the growth of a tumor.

Available assays	Manufacturer	Target	HPV genotypes	Genotyping	FDA approved
Cellular Assay					
CINtec	mtm Laboratories	p16ink4a			No
CINtec Plus	mtm Laboratories	p16ink4a/K1-67			No
Ki-67 (MIB1)	DakoCytomation	Ki-67			No

Gene Methylation as Biomarker

- **DNA methylation** is one of the epigenetic mechanisms that **influence gene transcription**, chromatin structure, genomic stability and …

- **Abnormal methylation of promoters of tumor suppressor genes is common in various cancers**, and the analysis of DNA methylation as a biomarker in clinical oncology seems to be promising.


Unmethylated
Methylated
Gene Expression
Gene
Gene Expression Repressed
Gene

Human Gene Methylation

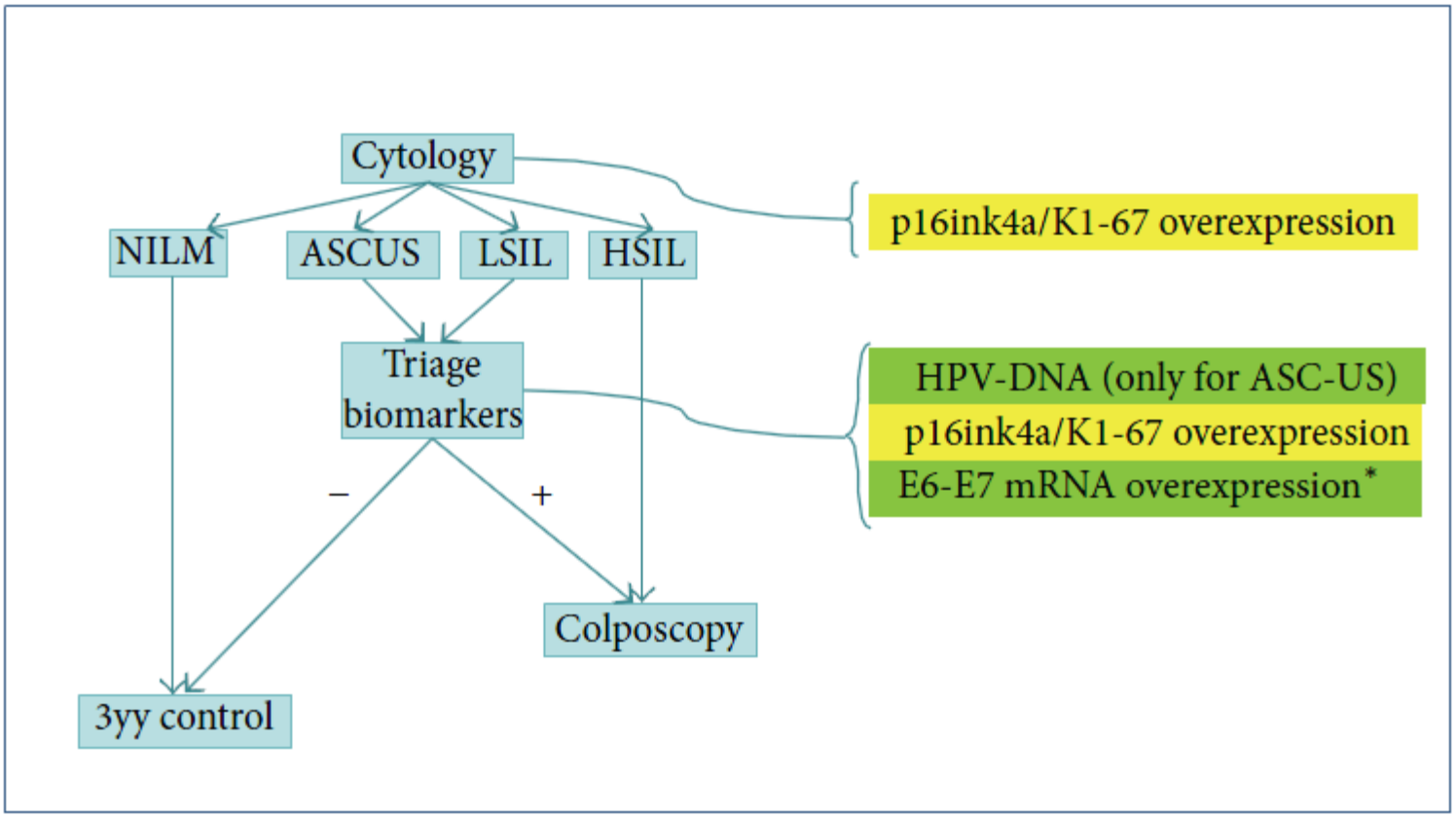
- In many cancers, tumor suppressor genes were found to be **inactivated by hypermethylation of their promoter region**.
- Therefore, detection of hypermethylation of tumor suppressor genes involved in cervical cancer genesis may provide powerful biomarkers for cancer detection. especially as methylation has been detected already at precancerous stages.
- **In fact, a recent studies are working on the CIN Grading and its related Gene methylation…**

Viral Gene Methylation

- The investigators found that elevated levels of DNA methylation on multiple CG sites in the L1, L2, E2, and E4 ORFs were significantly associated with CIN2 or worse after accounting for multiple testing.

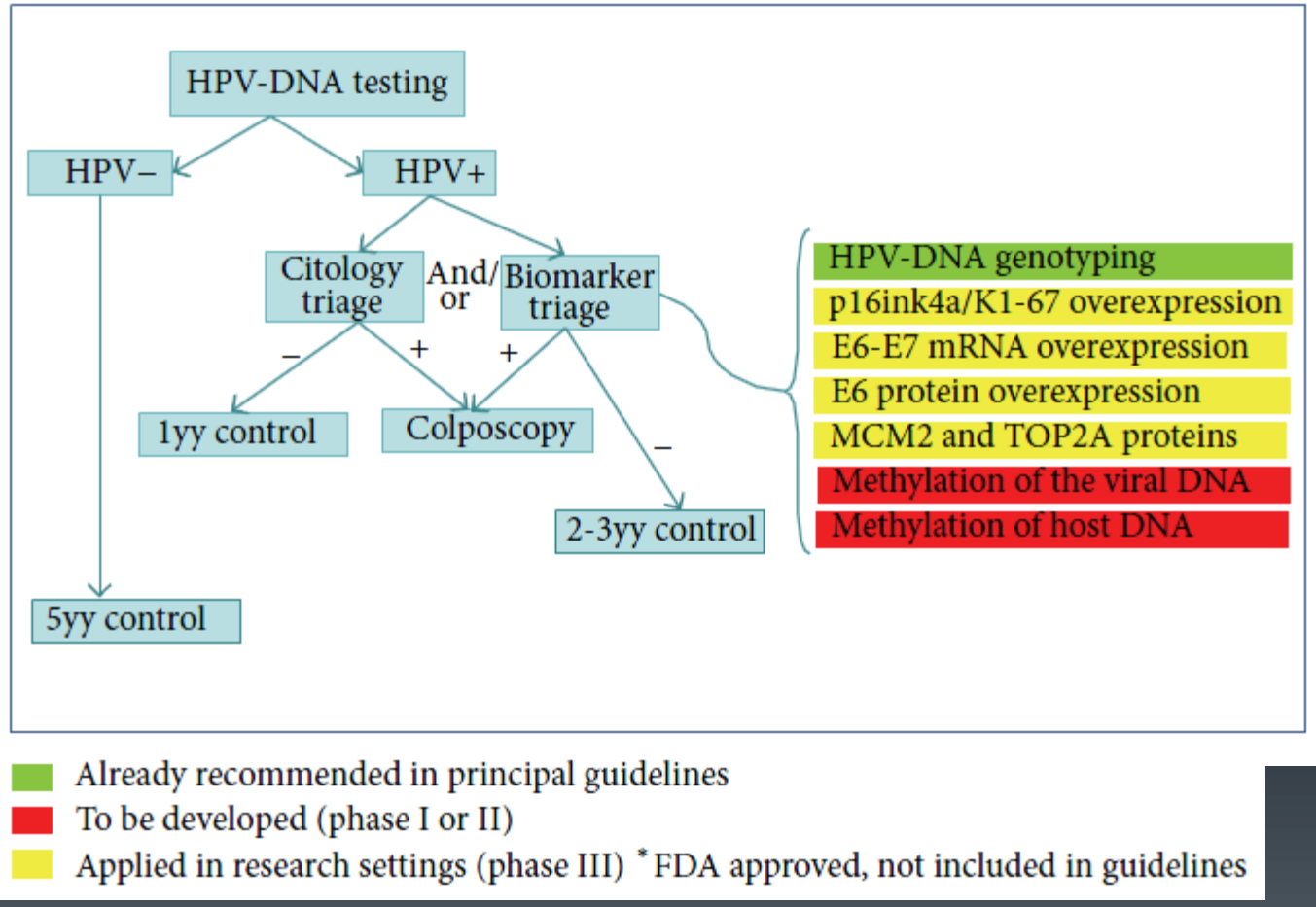
The direct relationship between methylation status of HPV L1 gene and diagnosis of CIN2 seems to be relatively consistent in most studies.

Screening strategy based on cytology



Maria Lina Tornesello et al. Viral and Cellular Biomarkers in the Diagnosis of Cervical Intraepithelial Neoplasia and Cancer. BioMed Research International. Volume 2013, Article ID 519619

Screening strategy based on HPV DNA tests



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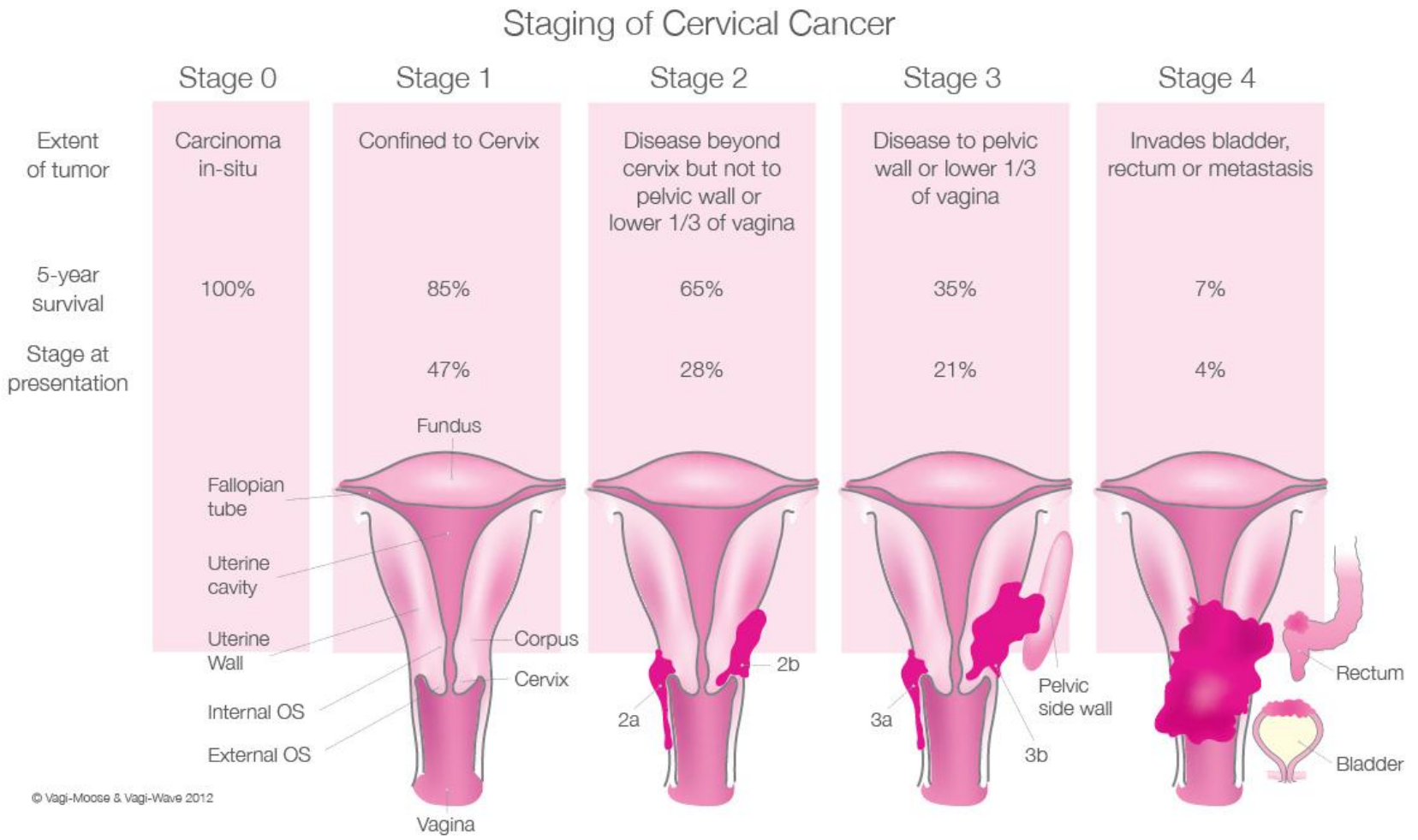
Cervical Cancer Stages



Treatment

Four types of standard treatment are used:

- **Surgery:** removing the cancer in an operation.
- **Radiation therapy:** use high-energy x-rays or other types of radiation to kill cancer cells or keep them from growing.
- **Chemotherapy:** use drugs to stop the growth of cancer cells, either by killing the cells or by stopping them from dividing
- **Targeted therapy:**use drugs or other substances to identify and attack specific cancer cells without harming normal cells

Human Papillomavirus Vaccines

- GARDASIL, Merck Co.
- June 2006 FDA approved
- Quadrivalent (4)
- composed of recombinant L1 protein-based viral like particles
- HPV 6, 11, 16 and 18
- 0, 2 and 6 months

- CERVARIX, GlaxoSmithKline
- October 2009 FDA approved
- Bivalent (2)
- contains recombinant L1 protein
- HPV 16 and 18
- 0, 1–2, and 6 months

FULL PAPER

BJC

British Journal of Cancer (2013) 108, 908–913 | doi: 10.1038/bjc.2013.22

Keywords: biomarkers; cervix; HPV

Comparing the performance of six human papillomavirus tests in a screening population

J Cuzick[1], L Cadman[1], D Mesher[1], J Austin[1], L Ashdown-Barr[1], L Ho[1], G Terry[1], S Liddle[2], C Wright[3], D Lyons[4] and A Szarewski*[,1]

[1]*Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK;* [2]*The Doctors Laboratory, 60 Whitfield Street, London W1T 4EU, UK;* [3]*Department of Cytopathology, St Mary's Hospital, Praed Street, London W2 1PG, UK and* [4]*Department of Gynaecology, St Mary's Hospital, Praed Street, London W2 1PG, UK*

Background: Several new assays have been developed for high-risk HPV testing of cervical samples; we compare six HPV tests in a screening population.

Methods: Residual material from liquid-based PreservCyt samples was assayed. Four tests (Hybrid Capture 2, Cobas, Abbott and Becton-Dickinson (BD)) measured HPV DNA while two used RNA (APTIMA and NorChip).

Results: Positivity rates ranged from 13.4 to 16.3% for the DNA-based tests with a significantly lower positivity rate for the Abbott assay. The Gen-Probe APTIMA assay was positive in 10.3% of women, which was significantly lower than all the DNA tests; the NorChip PreTect HPV-Proofer test was much lower at 5.2%. 40 CIN2+ cases were identified, of which 19 were CIN3+. All CIN3+ cases were HPV positive by all tests except for one, which was negative by the Abbott assay and five which were negative by the NorChip test.

Conclusion: All HPV tests except NorChip showed high sensitivity for high-grade lesions positive by cytology, suggesting co-testing is unnecessary when using HPV tests. Positivity rates in cytology-negative specimens were similar for the DNA-based tests, but lower for the APTIMA test suggesting this maintains the high sensitivity of DNA tests, but with better specificity.

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MATERIALS AND METHODS

Residual material was used from the liquid-based cytology PreservCyt samples from 6000 women who attended for a routine 3 or 5 yearly (depending on age) screening smear, and whose samples were sent to the cytology laboratory at St. Mary's Hospital, London. Unsatisfactory cytology samples were excluded; there were no other inclusion/exclusion criteria. Samples were linked to concurrent cytology results and any histology within 6 months of an abnormal smear, and this information was fully anonymised before being transferred for analysis.

Test	Sensitivity (95% CI)	Specificity (95% CI)
BD HPV		
CIN3 +	100.0 (82.4–100.0)	
CIN2 +	97.5 (86.8–99.9)	84.3 (83.3–85.2)
Roche Cobas		
CIN3 +	100.0 (82.4–100.0)	
CIN2 +	97.5 (86.8–99.9)	84.5 (83.6–85.4)
Qiagen Hybrid Capture 2		
CIN3 +	100.0 (82.4–100.0)	
CIN2 +	97.5 (86.8–99.9)	85.4 (84.5–86.3)
Abbott RealTime High Risk HPV		
CIN3 +	94.7 (74.0–99.9)	
CIN2 +	95.0 (83.1–99.4)	87.2 (86.3–88.0)
Gen-Probe APTIMA		
CIN3 +	100.0 (82.4–100.0)	
CIN2 +	97.5 (86.8–99.9)	90.2 (89.5–91.0)
NorChip PreTect HPV-Proofer[a]		
CIN3 +	68.8 (41.3–89.0)	
CIN2 +	71.4 (53.7–85.4)	95.2 (94.7–95.8)

CONCLUSIONS

In this evaluation of six HPV tests from residual liquid-based screening cytology specimens, all tests except for NorChip showed high sensitivity for high-grade lesions that were positive by cytology, suggesting that they are suitable for primary screening and that dual co-testing with cytology as well is unnecessary. Positivity rates in cytology-negative specimens were similar for the DNA-based tests, but were lower for the APTIMA test, suggesting it can maintain the high sensitivity of the DNA tests, but with a better specificity, so that fewer women would need triage tests or short-term follow-up. However, a long-term low-risk period after a negative test has yet to be demonstrated for APTIMA or any RNA-based test, as has been shown for some of the DNA-based tests, especially Hybrid Capture 2 (Dillner *et al*, 2008, Cuzick *et al*, 2008b, Mesher *et al*, 2010, Rijkaart *et al*, 2012). Direct demonstration of this is desirable to support its use in primary screening, The NorChip test had lower sensitivity but higher specificity, suggesting its role may be more in triage than primary screening.

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Thank You…